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CLINICAL STUDY

The *in vitro* activity of vaginal *Lactobacillus* with probiotic properties against *Candida*

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Abstract
Lactobacilli, the predominant vaginal microorganisms in healthy premenopausal women, control other members of the vaginal microflora and thus protect against bacterial vaginosis and urinary tract infections. It has been claimed that some lactobacilli are also protective against *Candida* vaginitis. Little is known, however, about the mechanisms by which these lactobacilli can control vaginal populations of *Candida* and prevent vaginitis. To address this question, vaginal *Lactobacillus* strains with known antagonistic properties against bacteria were tested for their cell surface properties, adhesion to vaginal cell lines *in vitro* and antagonistic activities against *Candida*. A small proportion of the lactobacilli tested adhered strongly to cultured vaginal epithelial cells and inhibited growth of *Candida albicans* but not of *C. pseudotropicalis*. This anticandidal activity was in some *Lactobacillus* strains related to hydrogen peroxide (H_2O_2) production, but catalase treatment did not suppress this activity in other *Lactobacillus* strains, suggesting alternative mechanism(s). Moreover, tested vaginal *Candida* strains were resistant to relatively high concentrations of H_2O_2 that markedly exceeded those produced by even the most active *Lactobacillus* strains.

Keywords: Lactobacillus, Candida, *cell surface properties, antagonistic activities, H_2O_2*

Introduction

Lactobacilli form a critically important component of the vaginal ecosystem, and are the predominant (10^7 to 10^8 CFU/g of vaginal fluid) vaginal microorganisms in healthy premenopausal women. Certain factors that increase the risk of bacterial vaginosis (BV) and urinary tract infections (UTIs) are associated with decreased vaginal *Lactobacillus* populations [1,2]. It has also been claimed that oral intake of *Lactobacillus acidophilus* is protective against *Candida* vaginitis (CV) [3]. The protective role that lactobacilli play in UTI and BV seems to be based upon two mechanisms, namely specific adherence by selected *Lactobacillus* species [4] to vaginal epithelium, leading to intensive colonization of this surface by formation of microcolonies and biofilm, and control of the remaining vaginal microflora by production of active metabolites, including acidic products, bacteriocin-like substances and hydrogen peroxide (H_2O_2) [1,5,6]. Little is known, however, about the mechanisms by which some *Lactobacilli* can control vaginal populations of *Candida* and thus prevent CV.

The aim of this study was to test vaginal *Lactobacillus* strains with known antibacterial effects for their cell surface properties, adhesion toward vaginal cell lines *in vitro* and antagonistic activities directed against yeasts, with special regard to H_2O_2 production.

Methods

Bacteria

A total of 111 *Lactobacillus* strains were randomly selected from a larger collection of strains with known antagonistic properties exerted against bacteria associated with BV, namely *Gardnerella vaginalis*, *Peptostreptococcus anaerobius* and *Prevotella bivia*, as described previously [7]. The strains were isolated from vaginas of healthy women attending the

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outpatient clinic for students in Cracow, Poland. All were premenopausal and were not menstruating at the time of collection. All of the women studied were clinically normal on vaginal examination, had a normal Nugent's score [8] and a low (4.0 to 4.5) pH of the vaginal fluid. They had neither received antibiotics nor used hormonal contraceptives during the previous 3 months. They presented a high level of personal hygiene and reported high-quality hygiene practices. They did not use vaginal douching. They were free of known STD as checked by routine methods. Informed consent was obtained from those agreeing to participate.

The strains were cultured on MRS agar [Oxoid, Basingstoke, UK]. They were initially identified using API 50 CHL (BioMerieux, Marcy l'Etoile, France) and then speciated using a polymerase chain reaction (PCR) technique and species-specific primers [9]. The strains were then stored on glass beads in MRS broth with 10% glycerol at -70°C, so that their genotypic and phenotypic properties were well preserved. Before the experiments, four to five glass beads coated with lactobacilli were transferred to 2 ml liquid MRS, then incubated at 37°C for 48 h in strictly anaerobic conditions using BioMerieux gas boxes with hydrogen and carbon dioxide gas generators [BioMerieux].

Surface properties

Hydrophobicity was estimated by the salt aggregation test (SAT) according to Jonsson and Wadström [10]. Bacterial cell suspensions were mixed with equal volumes of ammonium sulphate of various molarities (0.01 to 4.0 mol/l^{-1}). The lowest concentration of ammonium sulphate that produced visible aggregation was scored as the SAT hydrophobicity value. The aggregation in saline solution used as a control was regarded as auto-aggregation. Slime production was demonstrated using a qualitative method of Christensen et al [11]. The tested strain was grown in glass tubes in 3 ml MRS broth at 37°C for 24 h. After incubation, the culture was discarded and the tube filled again with 1 ml 0.1% solution of safranin [Poch, Glinerice, Poland] and rotated for 60 s. The staining solution was decanted, and the tubes inverted and dried at room temperature. The density of biofilm covering the tube walls was estimated using a semi-quantitative scale (- to + + +).

Adherence to vaginal cells

The ability of *Lactobacillus* strains to adhere to the human vaginal cell line A431 (kindly provided by Professor D. Dus, Polish Academy of Sciences) was checked by *in vitro* assay according to Gaffney et al., the degree of adhesion being evaluated according to a semi-quantitative score system (- to + + +) [12]. Briefly, a 24-h culture of A431 cells at 1 x 10^5 cells/ml, cultured in wells of a 12-well flat-bottom tissue culture plate (Iwaki, Fuuahashi, Japan) on Eagle's 1959 medium (MEM) with L-glutamine and sodium bicarbonate (IITD, Wroclaw, Poland) containing 5% fetal calf serum (Sigma-Aldrich, St Louis, USA) and antibiotics (penicillin 100 UI/ml, streptomycin 100 UI/ml, neomycin 200 μg/ml) (Sigma-Aldrich) was washed twice with PBS. Overnight cultures of bacteria were appropriately diluted with MRS + MEM (1:1) to give a concentration of approximately 10^8 CFU/ml, and was used to inoculate cells in wells; the pH of the mixture ranged from 5.0 to 5.6. After incubation at 37°C for 30 min, wells were washed four times with PBS to release unbound bacteria. The cells were then fixed with 3.7% formaldehyde for 1 h, washed twice with PBS and stained with crystal violet stain (Merck, Darmstadt, Germany), washed, dried and examined under the microscope (magnification x 100). According to our laboratory standard procedure, the adherent lactobacilli were counted in 20 random microscopic fields.

Antagonism against yeasts

From among the *Lactobacillus* strains, 14 strains showing most surface activity were compared, using a modified agar overlay method according to Fitzimmons and Berry [13], against *Candida albicans* and *Candida pseudotropicalis*. The yeast strains were freshly isolated from clinical cases of CV confirmed by physical examination and routine culture, identified phenotypically using API 20C AUX system (BioMerieux) and then stored at -70°C as described for lactobacilli. The examined *Lactobacillus* strains were cultured on agar plates containing 10 ml MRS, in the form of a stripe 2 cm wide across the plate. The plates were incubated in anaerobic conditions at 37°C for 24 h. The *Lactobacillus* growth was then overlaid with melted MYPG agar (Bacto YM Agar, Difco, Detroit, USA), MYPG with sodium thiocyanate (0.81 g/l) (POCH), MYPG with sodium thiocyanate (0.81 g/l) (POCH) and 20 μg/ml catalase (Sigma-Aldrich); the pH was adjusted to 6.2. After solidification of the agar overlay, overnight culture of *C. albicans* or *C. pseudotropicalis* in Sabouraud broth (Oxoid), diluted with PBS to 0.5 MacFarland's score, was streaked over the agar surface with a cotton swab. The plates were preincubated at 4°C for 4 h in a refrigerator, then kept at 37°C for 24 h in aerobic conditions and left for 24h at room temperature before reading. This culture method best displays the inhibition zones.

After adequate growth, the zones of inhibition of *Candida* were measured using a semi-quantitative scale on which (−) = lack of *Candida* growth inhibi-

tion over *Lactobacillus* culture (Figure 1); (+/−) = minimal inhibition of *Candida* growth over *Lactobacillus* culture; (+) = partial inhibition of *Candida* growth over *Lactobacillus* culture; (++) = total inhibition of *Candida* growth over *Lactobacillus* culture (Figure 2); and (+++) = total inhibition of *Candida* growth beyond *Lactobacillus* culture.

Semi-quantitative agar plate method for H_2O_2 detection

H_2O_2 production by the 14 selected strains of vaginal lactobacilli was examined using a method described by Eschenbach et al. [14], modified by Fontaine and Taylor-Robinson [15]. The examined *Lactobacillus* strains were cultured in a reductive manner on MRS plates containing 4 mg/ml 3-ethyl-benzthiazoline-6-sulfonic acid (ABSA) (Sigma-Aldrich) and 0.2 mg/ml horseradish peroxidase (HRPO, Sigma-Aldrich). The plates were then incubated in anaerobic conditions at 37°C for 48 h, after which they were exposed to air. The HRPO oxidised ABSA in the presence of H_2O_2, forming a blue pigment in and around the colonies. The first reading was performed after 1 h, the last after 24 h. The level of H_2O_2 produced was marked according to the intensity of blue coloration, and the results were calculated by a semi-quantitative scale on which (−) = lack of colony coloration; (+/−) = minimal blue colour change of the colony; (+) = small and limited blue colour change of the colony; (++) = large but incomplete blue colour change of the colony; and (+++) = clearly visible complete blue colour change of the colony.

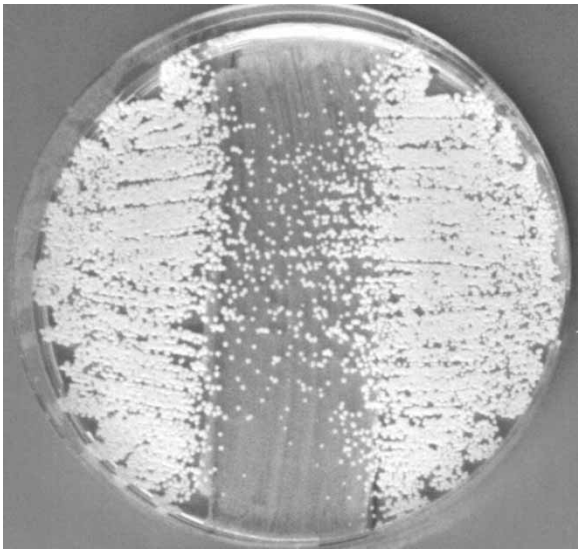

Figure 1. Example of agar plate method showing lack (−) of *Candida* growth inhibition over *Lactobacillus* culture.

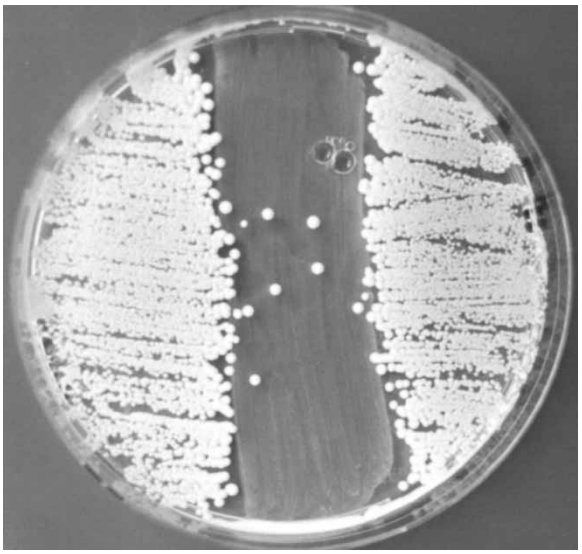

Figure 2. Example of agar plate method showing total inhibition (++) of *Candida* growth over *Lactobacillus* culture.

Analytical Merckoquant peroxide test strips for H_2O_2 production

Test strips (Merck) were used to measure the quantity of H_2O_2 on a detection scale between 0 and 100 mg/l. *Lactobacillus* strains were suspended in 1 ml liquid MRS and cultured at 37°C in anaerobic conditions for up to 48 h. The final density of *Lactobacillus* cells was estimated as approximately 1 x 10^9 CFU/ml. The test strip was then immersed in the culture and read against the scale provided by the producer 1 min after removal and several times during the incubation period.

Enzyme microimmunoassay test for H_2O_2 and O_2 production

The technique used for the measurement of H_2O_2 and O_2 production by *Lactobacillus* was an adaptation of Pick and Mizel's phenol red solution method[16] for testing H_2O_2 production by macrophages. *Lactobacillus* strains were suspended in 1 ml liquid MRS and cultured at 37°C in anaerobic conditions for up to 48 h. The final density of *Lactobacillus* reached approximately 1 x 10^9 CFU/ml. The cultures were centrifuged at 1200 rpm for 7 min, and a 50 μl sample of each supernate was placed in a well of a 96-well plate (Nunc, Rochester, USA). Following this, 50 μl of reagent 1 ((2 μl of phenol red) (Sigma), 2 μl of HRPO, 46 μl DPBS buffer and 10 μl 1N sodium hydroxide) were added. Absorbance of this mixture was measured at 600 nm. Control consisted of 50 μl DBPS buffer or MRS medium with 50 μl reagent 1 and 10 μl sodium hydroxide. The results, after deduction of control values, were calculated as nmoles or transformed into mg/l to compare with the test strips method. The tests were run in triplicate.

Effect of H_2O_2 on Candida *and* Lactobacillus

This was measured by incubating suspensions of randomly selected strains of *Candida* (approximately 10^7 CFU/ml) and *Lactobacillus* isolated from vaginal flora (approximately 10^8 CFU/ml) in PBS with decreasing concentrations of H_2O_2 in PBS. Clinical isolates of *Candida* and selected *Lactobacillus* species were used. Initial numbers of tested organisms were measured by counting colonies after serial tenfold dilutions on appropriate solid media (Sabouraud agar for *Candida* and MRS agar for *Lactobacillus*). The mixtures were incubated at 37°C for 1 h in an aerobic (*Candida*) or anaerobic (*Lactobacillus*) atmosphere. After this period, tenfold dilutions in PBS were prepared and plated on appropriate media. The inoculated media were cultured at 37°C for 24 h in the appropriate atmosphere and colonies were counted for CFU/ml. Concentrations of H_20_2 before and after incubation were controlled by the test strips method.

Results

The surface properties of *Lactobacillus* strains and their adherence to vaginal cells are shown in Table I.

Of the 111 tested vaginal *Lactobacillus* strains, most produced extracellular slime-like material, but only about 3% showed higher surface hydrophobicity. More than 50% of the tested vaginal *Lactobacilli* adhered to A431 line cells, although at different rates. Only a few strains were strongly adherent, whereas the rest showed moderate-to-low adhesion.

Lactobacillus strains showing high to moderate ability to adhere to vaginal cells ($n = 14$) were further tested for their anti-candidal activity. The selected strains were of different species, and although all of them inhibited growth of *C. albicans* to a certain degree, only five strains were distinctly active. This activity was inhibited by catalase incorporated into the test medium, whereas the anti-candidal properties of some less active *Lactobacillus* strains were not influenced. Addition of thiocyanate to test agar did not alter the degree of inhibition in any of the tested strains. Most lactobacilli were able at least slightly inhibit the growth of *C. pseudotropicalis* (Table II).

Only two *Lactobacillus* strains showed a positive reaction for H_2O_2 1 h after the exposure of the test plates to air, and these results were then confirmed

Table I Surface properties and adherence to vaginal cells of 111 human vaginal *Lactobacillus* strains.

Property	Number of strains	(%)
Salt agglutination		
+ + +	0	0
+ +	3	2.7
+	16	14.4
-	92	82.9
Slime production		
+ + +	23	21.7
+ +	37	33.3
+	28	23.3
-	23	21.7
Adherence		
+ + +	4	3.6
+ +	18	16.2
+	45	40.5
-	44	39.7

Salt aggregation test: + + +, 0.02M; + +, 0.05M; +, 0.5 to 1M; -. > 1M. Slime: + + +, intensive red colour of test tube; + +, red colour of test tube; +, pink colour of test tube; -, no colour of test tube. Adherence per 20 microscopic fields: + + +, > 100 bacteria; + +, 40 to 100 bacteria; +, 20 to 40 bacteria; -, < 20 bacteria.

Table II. Antagonistic properties of selected *Lactobacillus* strains against *Candida albicans* and *Candida pseudotropicalis*.

Strain number	Species	*C. albicans* MYGP	*C. albicans* MYGP + thiocyanate	*C. albicans* MYPG + thiocyanate + catalase	*C. pseudo-tropicalis* MYPG
11C	*L.fermentum*	+	+	+/−	+/−
151A	*L.rhamnosus*	+ + +	+ + +	+	+/−
41B	*L.rhamnosus*	+ +	+ +	+ +	+/−
17B	*L.fermentum*	+ +	+ +	+	-
30B	*L.plantarum*	+ +	+ +	+	+/−
113B	*L.plantarum*	+ + +	+ + +	+	+/−
41A	*L.fermentum*	+ + +	+ + +	+/−	+/−
6B	*L.plantarum*	+ +	+ +	+ +	+
G46B/3	*L.acidophilus*	+ + +	+ + +	+/−	+/−
G44B/1	*L.acidophilus*	+ +	+ +	+ +	+/−
G/2A	*L.acidophilus*	+ +	+ +	+	-
G/12A	*L.acidophilus*	+ +	+ +	+	-
G/26/2	*L.acidophilus*	+ + +	+ + +	+	-

Scale: -, lack of *Candida* growth inhibition over *Lactobacillus* culture; +/−, minimal inhibition of *Candida* growth over *Lactobacillus* culture; +, partial inhibition of *Candida* growth over Lactobacillus culture; + +, total inhibition of *Candida* growth over *Lactobacillus* culture; + + +, total inhibition of *Candida* growth beyond *Lactobacillus* culture.

by the more accurate microassay. Oxygenation prolonged to 24 h increased the number of H_2O_2-producing lactobacilli, but these results were inconsistent with the microassay and thus unspecific. The test strips method detected H_2O_2 in only two (most active) strains at levels corresponding to those obtained at microassay. The amounts of H_2O_2 produced by the most active strains varied from 2 to 30 mg/l in the test strips method and from 1.5 to 28.7 mg/l in the microassay. However, some discrepancies between H_2O_2 levels and degrees of agar coloration were noticed in several strains (Table III).

Selected *Candida* strains belonging to different species were resistant to relatively high concentrations of H_2O_2 in test conditions. Concentrations of 30 g/l were necessary to kill all *Candida* cells; 3 g/l was microbicidal for only a minor proportion of *Candida* cells. No differences among the strains were observed, and concentrations of H_2O_2 did not change during the incubation period (Table IV).

*Lactobacillus s*trains producing as well as those not producing H_2O_2 were equally sensitive to 3 g/l and higher concentrations of H_2O_2 except for *L. acidophilus* G/12A and *L. delbrueckii* 117A, which were partially inhibited by the lowest tested concentration (150 mg/l) (Table V).

Discussion

It is generally believed that vaginal *Lactobacillus* strains control the vaginal microflora, including *Candida albicans* [6], by colonizing the vaginal epithelium and inhibiting the growth of the other microorganisms. Therefore *Lactobacillus* strains, as candidates for vaginal probiotics, are usually tested *in vitro* for their ability to adhere to the vaginal epithelium, their antimicrobial activity towards *Gardnerella vaginalis* and *C. albicans*, and their production of H_2O_2 which, according to several authors, may be mainly responsible for inhibitory activity [1,17].

Unlike other workers [18], in this study as well as in our previous experiments on *Lactobacillus* adherence *in vitro* [4] we used the human vaginal cell line A431. This line is derived from vaginal epithelial epidermoid carcinoma. It is useful for standardizing tested bacterial adherence to vaginal cells, to enable comparison of different research approaches in this

Table III. Hydrogen peroxide (H_2O_2) and superoxide anion production by selected *Lactobacillus* strains.

		H_2O_2 measurement			
Strain number	Species	Plate method, 1h	Plate method, 24h	Test strips, (mg/l)	Microassay (mg/l)
2B	*L.plantarum*	-	-	0	0
11C	*L.fermentum*	-	-	0	0
101A	*L. rhamnosus*	-	-	0	0
41B	*L. rhamnosus*	-	+/−	0	0
17B	*L. fermentum*	-	+/−	0	0.5
30B	*L. plantarum*	-	+ + +	0	0.7
113B	*L. plantarum*	-	+/−	0	0.9
41A	*L. fermentum*	-	+/−	0	0.5
6B	*L. plantarum*	-	+	0	0
G46B/3	*L. acidophilus*	-	+	0	0
G44B/1	*L. acidophilus*	-	+	0	0
G/2A	*L. acidophilus*	-	+/−	0	0
G/12A	*L. acidophilus*	+ +	+ + +	2.0 to 20.0	1.5 to 17.2
G/26/2	*L. acidophilus*	+ +	+ + +	3.0 to 30.0	2.0 to 28.0

*Scale: -, lack of colony coloration; +/−, minimal blue colour change of the colony; +, small and limited blue colour change of the colony; + +, large but incomplete blue colour change of the colony; + + +, clearly visible, complete blue colour change of the colony.

Table IV. Susceptibility of tested *Candida* strains to different concentrations of hydrogen peroxide (H_2O_2).

		Candida density after exposure to decreasing concentrations of H_2O_2 (CFU/ml)			
Candida strains	Initial *Candida* density (CFU/ml)	3% (30 g/l, 882.4 mM)	0.3% (3 g/l, 88.24 mM)	0.03% (300 mg/l, 8.824 mM)	0.015%(150 mg/l,4.4 mM)
C. albicans, IM 145	$3.0x10^6$	0	$1.0x10^5$	$6.7x10^5$	$1.5x10^6$
C. albicans, IM 267	$1.3x10^7$	0	$1.2x10^5$	$1.0x10^7$	$1.3x10^7$
C. glabrata, IM 078	$5.2x10^7$	0	$6.0x10^6$	$3.0x10^7$	$3.8x10^7$
C. crusei, IM 335	$1.2x10^7$	0	$3.2x10^5$	$7.0x10^6$	$1.0x10^7$

Table V. *Susceptibility of selected *Lactobacillus* strains to different concentrations of hydrogen peroxide (H_2O_2).

Lactobacillus strains	Initial *Lactobacillus* density (CFU/ml)	*Lactobacillus* density after exposure to decreasing concentrations of H_2O_2 (CFU/ml)			
		3%, 30 g/l, 882.4 mM	0.3%, 3 g/l, 88.24 mM	0.03%, 300 mg/l, 8.824 mM	0.015%, 150 mg/l, 4.4 mM
L.plantarum 30B	$2.0x10^8$	0	0	$1.0x10^8$	$1.6x10^8$
L. fermentum 17B	$4.0x10^9$	0	0	$2.0x10^8$	$2.8x10^8$
L. rhamnosus 41B	$4.3x10^7$	0	0	$2.0x10^7$	$2.5x10^8$
L. acidophilus G/12A	$9.0x10^7$	0	0	$1.0x10^4$	$3.0x10^6$
L. delbrueckii, var. delbrueckii 117A	$4.9x10^8$	0	0	$1.6x10^7$	$2.7x10^7$

*Density as mean of CFU/ml calculated from three measurements.

area. Using A431 cells, we found that only a minor proportion of the tested vaginal *Lactobacillus* strains showed a distinct degree of adherence, in spite of securing test conditions that resembled as much as possible the vaginal milieu (the pH of the reaction mixture during the test was kept at 5.0 to 5.6).

By means of a screening agar technique designed to study interactions between selected vaginal *Lactobacillus* strains and yeasts, we also demonstrated that only a few lactobacilli were able to inhibit *C. albicans* growth. Although lactic acid production seems to be a major factor in maintaining low vaginal pH and controlling strictly anaerobic bacterial vaginal flora [7,19], it is evident that this product, common to all lactobacilli, cannot be selective for acid-tolerant vaginal yeasts [20]. Our test system, based on a double layer of two media, had a low pH (6.2) initially, which became even more acidic (pH = 4.8) during incubation because of sugar fermentation by both lactobacilli and *Candida*.

Inhibition of *Candida* growth by single strains of lactobacilli *in vitro* was been noted some decades ago [21] and also demonstrated *in vivo* [22]. However, the mechanism of this growth inhibition has not been fully elucidated. The importance of H_2O_2 production by lactobacilli, proposed as vaginal probiotics and indicated for prevention of CV, is usually stressed [13,14]. In our studies, only some of the *Lactobacillus* strains showing inhibitory properties against *Candida* produced H_2O_2 in amounts detectable by the screening plate method commonly used in previous studies [14]. Moreover, results obtained by the more exact quantitative method confirmed that only two strains produced H_2O_2 in amounts exceeding 20 mg/l. To check this possibility, for several *Candida* strains we estimated minimal microbicidal concentrations of H_2O_2 which ranged from about 0.3% to 0.03% (3 to 0.3 g/l). These values markedly exceeded concentrations produced by tested lactobacilli. This does not support the hypothesis that for the direct inhibition of several *Candida* strains H_2O_2 is solely responsible. However, successful inhibition of *Candida* by vaginal lactobacilli producing H_2O_2 may occur *in vivo*, since peroxide interacts with peroxidases and halides present in vaginal secretions [23]. Moreover, it may be hypothesized that H_2O_2 interacts *in vivo* with ferrous ions present in vaginal secretions, especially during menstruation, to form highly reactive hydroxyl radicals via the Fenton reaction [24].

Catalase markedly diminished the inhibitory activity of the two most active lactobacilli producing H_2O_2, and also the activities shown by other strains that did not produce H_2O_2 in all three tests. This may suggest that the enzyme interferes with other products of lactobacilli with anti-candidal activity.

Fitzsimmons and Berry [13] postulated that anti-candidal activity is based on the production of hypothiocyanate from H_2O_2. However, this possibility has not been confirmed in our observations, since addition of thiocyanate to the test medium had no influence on the degree of inhibition.

Several of our *Lactobacillus* strains that were inhibitory to *C. albicans* showed that their activity was probably independent of H_2O_2 production. Sookkhee *et al.* [25] recently found that the anti-candidal activity of some oral *Lactobacillus* strains was not inhibited by heat treatment, which also suggested alternative mechanisms of inhibition. In fact, several antifungal substances such as cyclic dipeptides, pyroglutamic acid and other substances of low molecular mass, such as lactones, produced by different *Lactobacillus* species, have recently been characterized [19,26].

Conclusions

In the light of our experiments it seems that anti-candidal activity of vaginal *Lactobacillus* isolates is related to various overlapping mechanisms. Production of H_2O_2 does not seem to be a major mechanism in the direct inhibition caused by lactobacilli, and it certainly is not the sole active inhibitory product.

Certain strains of *Lactobacillus* genus, isolated from the vagina and representing major species colonizing vaginal mucosa of healthy women, express distinct

adherence and antagonistic properties *in vitro* which may indirectly indicate their protective role in stabilizing the vaginal microflora and controlling other members of the vaginal flora, including *Candida albicans*. It seems that inhibition of *C. albicans* by different *Lactobacillus* strains is dependent on various mechanisms.

Although maintenance or reconstruction of the normal composition of the vaginal microflora by applying properly selected lactobacilli may be of prophylactic value in preventing various genitourinary system infections in women, including CV, more studies based on well standardized methods of testing candidates for vaginal probiotics are necessary, before entering the stage of clinical studies. The *in vitro* tests used by us could be useful in the selection of candidate *Lactobacillus* strains for vaginal probiotics.

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